

Rec'd

DEC 2002

10/517382

PCT/OK 03/00384

REC'D 01 JUL 2003

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PA 991637

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APPLICATION NUMBER: 60/387,407

FILING DATE: June 11, 2002

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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

06/11/02  
JCS49 U.S. PTO

11040 U.S. PTO  
60/387407  
06/11/02

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<input type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (280 characters max) A DISPOSABLE CARTRIDGE FOR CHARACTERIZING PARTICLES SUSPENDED IN A LIQUID					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input type="checkbox"/> Customer Number				Place Customer Number Bar Code Label here	
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ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/>	Specification	Number of Pages	31	<input type="checkbox"/>	CD(s), Number
<input checked="" type="checkbox"/>	Drawing(s)	Number of Sheets	11	<input type="checkbox"/>	Other (specify)
<input type="checkbox"/>	Application Data Sheet. See 37 CFR 1.76				
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)					
<input checked="" type="checkbox"/>	Applicant claims small entity status. See 37 CFR 1.27.				FILING FEE AMOUNT (\$)
<input checked="" type="checkbox"/>	A check or money order is enclosed to cover the filing fees				
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<input type="checkbox"/>	Payment by credit card. Form PTO-2038 is attached.				\$80.00
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
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<input type="checkbox"/>	Yes, the name of the U.S. Government agency and the Government contract number are:				

Respectfully submitted,

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06/11/2002

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ALB.009P

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P18SMALL/REV05

**A Disposable Cartridge for Characterizing Particles Suspended in a Liquid**  
**FIELD OF THE INVENTION**

5 The present invention relates to a disposable cartridge for characterizing particles suspended in a liquid, especially a self-contained disposable cartridge for single-use analysis, such as for single-use analysis of a small quantity of whole blood. The self-  
10 contained disposable cartridge facilitates a straightforward testing procedure, which can be performed by most people without any particular education. Furthermore, the apparatus used to perform the test on the cartridge, could be made simple, light and maintenance free, thus giving full portability and a large range of operation for the user. The invention provides steps for pre-analytic handling of samples such as hemolysing of red blood cells and inactivation of coagulation of blood platelets.

**BACKGROUND OF THE INVENTION**

15 Present instruments for particle characterization such as counting and sizing are fairly expensive, immobile and require operation by trained personnel. The consequence hereof has been that many instruments are placed in dedicated laboratories that are operated by specialized personnel. Furthermore, the samples to be analyzed must be transported to this laboratory and the results are reported back to the requiree.

20 In WO 01/11338, which is hereby incorporated by reference, an apparatus is disclosed for characterizing particles suspended in a liquid, comprising a disposable cartridge and a docking station for removably receiving the cartridge. The cartridge comprises a housing with a collection chamber bounded by a wall containing an orifice for the passage of the particles and having an inlet/outlet for connection to a source of positive or negative gas pressure, and components of a particle  
25 characterization device for characterizing particles passing through the orifice that are connectable from outside the housing. The docking station comprises a port for connection with a source of positive or negative gas pressure and forming a gas connection with the inlet/outlet when the cartridge is received in the docking station, and means for operative connection with the components of a particle characterization device when the cartridge is received in the docking station.

30 In PCT/DK02/00292, which is hereby incorporated by reference, a device for sampling a small and precise volume of liquid is disclosed, comprising a movable member with a cavity for entrapment and displacement of an accurate part of a liquid sample.

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It is a disadvantage of these prior art devices that several devices are used to perform an analysis, e.g. of a whole blood sample. The sample taking is performed with a separate device, and the sample has to be transferred to another device for sample preparation before it is finally transferred to a sensor for analysis.

- 5 In WO 99/01742 a disposable sampling device is disclosed for an apparatus for counting particles contained in a liquid. The sampling device is connectable in a defined position to the apparatus. The device has means for introducing a sample therein, means for metering a defined volume of the sample, means containing a defined volume of a diluting liquid, a diluting chamber, means for simultaneously  
10 directing the defined volume of sample and the defined volume of diluting liquid to the diluting chamber for obtaining therein a diluted sample, means for directing at least a portion of the diluted sample past particle counting means and signal transmitting means connecting the particle counting means and terminal means located at an outer boundary of the housing in a position corresponding to a location of terminal  
15 means of the apparatus when the housing is connected thereto in the defined position.

- During blood analysis with the device described in WO 99/01742, the blood sample is pumped back and forth several times for dilution, mixing and analysis, and the flow system is closed so that the pressure in the system is increased and decreased  
20 above and below, respectively, atmospheric pressure during movement of the sample. Further, sample taking requires pumping with a membrane or another flow actuator causing entrance of blood into the flow system of the device. Thus, the above disclosed flow system is rather complicated.

- The particle counting is, as described in WO 99/01742, performed in an open ended  
25 tube so that the volume of diluted sample passing the particle counting sensor is very small.

- The blood analysis, as described in WO 99/01742 does not take into account that particles of different kind and concentration might need pre-analytic separation, decomposition, staining or labeling in order to be accurately recorded by the sensing  
30 principle in account.

The blood test sequence, as described in WO 99/01742 does not take into account that users without prior education herein, should be able to learn how to perform this test themselves, i.e. no pre-analytical dilution steps should be required.

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**SUMMARY OF THE INVENTION**

Thus, it is an object of the present invention to provide a cartridge for characterizing particles suspended in a liquid that enables sample taking, sample preparation, and particle characterization so that analysis may be performed within one device without a need for sample handling and sample transfer to another unit.

It is a further object of the present invention to provide a cartridge that is adapted for single-use to be discarded after analysis of one liquid sample.

It is another object of the present invention to provide a cartridge that has a simple flow system.

It is yet another object of the present invention to provide a flow system in the cartridge communicating with the surroundings so that the pressure in the flow system remains substantially constant at atmospheric pressure.

It is yet still another object of the present invention to provide a flow system allowing a relatively large volume of diluted sample to pass the particle characterizing means.

According to the present invention, the above-mentioned and other objects are fulfilled by a cartridge for characterizing particles suspended in a liquid, comprising a housing with a mixing chamber and a collection chamber separated by a wall containing an orifice for passage of the particles between the mixing chamber and the collection chamber. Particle characterization means are provided for characterizing particles passing through the orifice.

Sample taking may be performed through a first opening in the outer surface of the housing for entrance of a liquid sample into a first cavity in the housing. The housing further comprises a sampling member that is movably positioned in the housing. The sampling member has a second cavity for receiving and holding a small and precise volume of liquid. A third opening in the sampling member forms an input to the second cavity. In a first position of the sampling member, the second opening is in communication with the third opening for entrance of the liquid sample into the second cavity, and, in a second position of the sampling member, the third opening is in communication with an inlet to the mixing chamber.

Thus, the sampling member operates to receive and hold a precise volume of liquid sample and to transfer the sample to the inlet of the mixing chamber.

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Preferably, liquid to be sampled enters the cavities by capillary attraction causing a liquid flow. Utilization of capillary forces simplify the flow system, since no pumps, membranes, syringes or other flow generating means are, in contrast to WO 99/01742, needed to take the sample.

- 5 Thus, the first cavity may form a first capillary tunnel for entrance of a liquid sample by capillary attraction. The capillary tunnel is dimensioned so that, upon contact between the first opening and liquid to be sampled, a sample of the liquid is drawn into the first opening and the first capillary tunnel and the second opening by capillary attraction.
- 10 Further, the second cavity may form a second capillary tunnel adapted for drawing the liquid sample into the second cavity by capillary attraction. Preferably, the first and second capillary tunnel has the same diameter, and it is also preferred that, in the first position, the first and second capillary tunnel extend along substantially the same longitudinal center axis.
- 15 Preferably, the sampling member is rotatable about an axis of rotation that is substantially perpendicular to a longitudinal axis of the second cavity.
- Additionally or alternatively, the sampling member may be displaced in a direction substantially perpendicular to a longitudinal axis of the second cavity.
- 20 The surface of the first and second inner capillary tunnel walls may be hydrophilic whereby the capillary attraction of the liquid sample is facilitated. For example, the inner tunnel walls may be made of e.g. glass or polymers, such as polystyrene.
- Alternatively, the capillary tunnel walls may be made of another type of material and covalently or non-covalently coated with a hydrophilic material, such as a polymer or a reagent.
- 25 The capillary tunnel may also include one or more reagents adhered or chemically bonded to the inner tunnel wall. These reagents serve the purposes of further facilitating the capillary attraction of the sample and optionally also causing a chemical reaction in the liquid sample, e.g. introducing anticoagulant activity in a blood sample. Such reagents may comprise heparin, salts of EDTA, etc.
- 30 Preferably, the sampling member is made of a polymer.

In accordance with a further aspect of the invention, an apparatus is provided for characterizing particles suspended in a liquid, comprising a cartridge as disclosed

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herein, and a docking station for removably receiving the cartridge, the docking station comprising connectors for operational connection with the particle characterization means when the cartridge is received in the docking station.

5 The cartridge may further comprise a cartridge port communicating with the collection chamber for causing a liquid flow through the orifice, and the docking station may further comprise a corresponding port for forming a gas connection with the cartridge port when the cartridge is received in the docking station for application of a pressure causing a liquid flow through the orifice.

10 The particle characterization means may include a first electrode in the mixing chamber and a second electrode in the collection chamber, each electrode being electrically connected to a respective terminal member accessible at the outer surface of the cartridge for operational connection to the respective connector of the docking station when the cartridge is received in the docking station. Generally, it is preferred that all necessary electrical and fluid connections to the cartridge can be  
15 established by fitting the cartridge into the docking station, preferably by a simple push fit.

The first and second electrodes may facilitate particle characterization utilizing the well-known Coulter impedance principle, e.g. for counting and sizing of blood cells. This method has become a globally accepted method and is being used in the  
20 majority of haematology-analysers. Several thousand particles per second may be characterized with high precision and accuracy utilizing this principle.

With the electrical impedance technique it is possible to resolve the particle volume from the measurement. By maintaining a constant current across the orifice, the recorded voltage pulse from particles displacing the electrolyte in the orifice will have  
25 a height proportional to the volume of the particle. This is because particles can be considered non-conducting compared to the electrolyte, the electrical field (DC or RF) in the center of the orifice is homogeneous, which is normally the case when the diameter is smaller than the length of the orifice ( $l/D > 1$ ), the particle is to be considered small compared to the diameter of the orifice ( $d < 0.2 \cdot D$ ), only one particle  
30 passes through at a time and the particles are passed through the orifice in the axial path.

Normally such apparatus is operated so that the flow through the orifice is into the collection chamber.

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Preferably, the length of the orifice or passageway is from 1 to 1000  $\mu\text{m}$ , for example about 50  $\mu\text{m}$ . Desirably the axial length of the passageway is chosen such that only one particle will be present in the passageway at the time when detecting particles of from 0.1 to 100  $\mu\text{m}$  diameter. However, considerations to the homogeneity of the electrical field in the passageway may require a length of the aperture larger or equal to the diameter. The counts, of which some may be simultaneous counting of two particles, can be corrected mathematically by implementing a statistical estimation. The aspect ratio of said passageway, (axial length divided by diameter) is preferably from 0.5:1 to 5:1, more preferably from 1:1 to 3:1.

10 Preferably, the largest cross-sectional dimension of said passageway at said common location is from 5 to 200  $\mu\text{m}$ , for example 10 to 50  $\mu\text{m}$ .

As explained above, the present invention provides in preferred aspects a sensor based on a membrane fabricated in e.g. a polymer sheet by laser ablation. The membrane has an orifice placed relatively in the center of the membrane, which can be used for aspiration of particles suspended in a liquid, as the sensor is submerged into the liquid. This way of transporting particles into a measuring region is known for electrical characterization of particles by the Coulter principle (V. Kachel, "Electrical Resistance Pulse Sizing: Coulter Sizing", Flow Cytometry and Sorting, 2. ed., pp 80, 1990 Wiley-Liss, Inc.).

20 The cartridge may further comprise a breather inlet/outlet communicating with the surroundings for preservation of substantially ambient atmospheric pressure in the cartridge flow system for facilitation of liquid flow through the orifice.

Preferably, the cartridge is designed to be disposable after a single use. It is desirable that after use there is no need to clean the apparatus before it can be used in a new assay procedure with a new cartridge. Accordingly, escape of liquid from the cartridge at its entry into the docking station should be avoided. To this end the positioning of the orifice with respect to the breather inlet/outlet, the second chamber inlet/outlet and the particle characterization device components is preferably such that a volume of liquid sufficient for the desired particle characterization can be drawn or pumped through the orifice without the liquid passing out of the housing. Generally, it should be possible to pass a volume of liquid which is at least 0.1 ml to 10 ml, e.g. 5 ml, through the orifice whilst particle characterization measurements are being made with no liquid leaving the cartridge.



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The cartridge may comprise volume metering means for determining the beginning and end of a period during which a predetermined volume of liquid has passed through the orifice.

5 Preferably, the volume metering means comprises a volume metering chamber with an input communicating with the collection chamber and an output, and wherein presence of liquid is detected at the input and at the output, respectively.

For example, presence of liquid may be detected with a secondary electrode positioned at the input and a further secondary electrode positioned at the output, or presence of liquid may be detected optically, etc.

10 It is preferred that the input and output of the metering chamber is formed by narrow channels for accommodation of only a small liquid volume compared to the volume of the metering chamber so that the actual positioning of the volume metering means, e.g. secondary electrodes, in the channels do not substantially influence the accuracy of the volume metering means determination.

15 The mixing chamber or the collection chamber may constitute the volume metering chamber, however, it is preferred to provide an independent volume metering chamber facilitating positioning of the volume metering means, e.g. the secondary electrodes.

20 The volume metering means may be positioned for sensing when liquid in the metering chamber is at or above respective levels in the volume metering chamber.

Preferably, the volume metering means are connected to respective terminal members accessible from the exterior of the cartridge, and the docking station preferably comprises corresponding connector members for connecting to the respective terminal members.

25 The volume metering means may be used for sensing when the level of the liquid is such that the respective metering means are or are not immersed in the liquid and may therefore serve for determining the beginning and end of a period during which a fixed volume of liquid has passed through the orifice. For example, particle characterization may begin when the level of the liquid just falls below the level of a  
30 first metering means and may end when the level of the liquid just falls to below a second metering means, the volume of liquid passing through the orifice during this period being defined by the separation of the respective metering means.

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Where the end point of the passage of a defined volume of liquid is the effective emptying of one chamber to below the level of the orifice, it is preferred that each of the collection and mixing chambers (or at least that chamber from which liquid passes) has a transverse cross sectional area at the level of the orifice which is substantially less than the transverse cross sectional area of the chamber over a substantial part of the height of the chamber above the orifice.

According to a further aspect of the present invention a method is provided of operating a particle characterization apparatus comprising a cartridge as disclosed herein, the cartridge being demountable from the apparatus, the method comprising sampling liquid containing particles with the cartridge through the first opening with the sampling member in its first position, positioning the cartridge in the apparatus, moving the sampling member to its second position, pumping liquid in the storage chamber through the second cavity and into the mixing chamber together with the liquid sample, making particle characterizing measurements, disconnecting the cartridge from the apparatus, and discarding the cartridge.

Generally, in all embodiments it is preferred that all components which are wet by the sample in use are disposable and all non-disposable components can be re-used without cleaning.

It is an important advantage of the present invention that means for liquid sample preparation and analysis are integrated into a disposable cartridge. For example, the analytical steps comprise sampling of a precise amount of blood, dilution of said amount of blood and finally mixing the blood with diluent into a homogeneous solution. The analysis may include spectrophotometric analysis of the liquid.

Thus, according to the present invention, means are provided for unambiguously making a blood analysis, such as counting the blood cells in a small amount of blood coming from a droplet of capillary blood. Means are provided for taking an exact amount of blood sample, reagents present in the diluent may be added for e.g. dilution and/or chemical preparation of the sample, and the mixed sample and diluent flows through a sensor for analysis of individual blood cells and determination of the volume of the analyzed quantum of liquid.

As a supplement a spectrophotometric measurement can be performed in order to quantify the content of e.g. hemoglobin.

The cartridge may comprise the following parts:

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1. A liquid storage chamber
2. A blood sampling device
3. A mixing chamber
4. A flow through sensor arrangement
- 5 5. A collection chamber
6. A volume metering arrangement comprised of a chamber and two connected flow channels
7. A hydraulic connection for moving the liquid through the cartridge

10 The concept of the disposable unit can be further combined with the following additional parts:

- A. Electrodes for liquid level measurement
- B. Optical structures for optical liquid level measurement
- C. Anti-coagulation treatment of surfaces
- D. Reagents in the diluent for modification of e.g. blood-cells
- 15 E. Mixing flue or baffle for assisted mixing
- F. Multiple volume metering arrangements for altering volumes
- G. A coating tape covering the sample inlet before use
- H. A waste chamber for waste/overflow
- I. A valve preventing liquid to exit through exhaust tube
- 20 J. A piston or membrane instead of the vacuum tube
- K. A window for spectrophotometric measurements

25 The liquid storage chamber (part 1) holds the required amount of diluent used for the blood analysis. When the blood has been sampled into the cartridge, the diluent is flushed through the capillary to wash out the sampled blood and dilute it as required by the test. Dilutions of 100 to 10.000 times are considered to be normal ratings. The liquid storage chamber should preferably be constructed to facilitate total draining of the chamber. This would be accomplished by having a slanting of the bottom of the chamber.

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The sampling unit (part 2) may comprise a capillary partly penetrating through a movable rod placed in a tight-fitting supporting body. The movable rod is used for entrapment of a precise amount of blood sample. When blood has filled the capillary by capillary forces, the rod is turned and/or displaced from its initial position in the supporting body, thus isolating the part of the capillary that extends through the rod.

After moving the rod in the supporting body into its second position the capillary forms a liquid path between the liquid storage chamber and the mixing chamber (part 3). By evacuation of the mixing chamber the diluent and blood sample is forced to pour into the mixing chamber, where mixing will be performed by convection.

The flow through sensor arrangement (part 4) is comprised of a small orifice in a membrane that establishes a liquid path from the mixing chamber to the collection chamber. On either side of the membrane (in the mixing chamber and in the collection chamber) two electrodes are placed contacting the liquid.

The collection chamber (part 5) forms a liquid priming function of the backside of the sensor system.

The volume metering system (part 6) is necessary for determination of the cell concentration. It comprises volume metering chamber of a known volume with two relatively thin channels connecting the inlet at the bottom and the outlet at the top. Sensing of the liquid at the inlet and outlet can be applied by optical or electrical means.

The outlet of the volume metering system is connected through a channel (part 7) to a source of pressure for moving the liquid through the cartridge.

The additional parts to the concept are further described here:

Addition A: Two electrodes for liquid level measurement are connected through the body of the cartridge into the inlet and outlet of the volume metering cell respectively. The electrodes will be short-circuited through the saline liquid to the electrode placed in the collection chamber, which can be registered through an external electrical arrangement.

Addition B: Instead of integrating electrodes into the cartridge external optical sensors can be used. The surface over the inlet and outlet of the volume metering cell should be structured to optimize the coupling of the light into the channel. The presence of liquid in the channel will result in a transmission of the signals as opposed to a reflection, which can be registered by optical sensors.

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Addition C: The anti-coagulation treatment of surfaces in the sampling structure can be achieved by having selected compounds adhered or chemically bonded to these surfaces. Examples of such compounds are heparin and salts of EDTA.

5 Addition D: Reagent in the diluent for modification of e.g. blood-cells. This reagent can consist of one or several compounds capable of hemolysing the erythrocytes. In addition other compounds may be added in order to: stabilize leukocytes and/or thrombocytes, adjust the pH-value and osmotic pressure, minimize bacterial growth, modify the hemoglobin present and minimize batch-batch variations. The following examples have been included to provide information on relevant subjects related to  
10 the performance of a self-contained test cartridge.

Examples of compounds capable of selectively hemolysing the red blood cells are: mixtures of quaternary ammonium salts as described in e.g. US 4,485,175; US 4,346,018; US 4,745,071; US 4,528,274; and US 5,834,315.

15 Examples of compounds capable of, during the hemolysis of the red blood cells, stabilizing the leukocytes are N-(1-acetamido)iminodiacetic acid, procaine hydrochloride as described in e.g. US 4,485,175 and 1,3-dimethylurea as described in e.g. US 4,745,071. In addition N-(1-acetamido)iminodiacetic acid is proposed to further assist the quaternary ammonium salts in minimizing debris stemming from hemolysed red blood cells as described in e.g. US 4,962,038 and adjust the pH-value  
20 (see below).

Examples of compounds added in order to adjust the pH-value and not least importantly the osmotic pressure of the diluent are: N-(1-acetamido)iminodiacetic acid, sodium chloride, sodium sulphate as described in e.g. US 4,485,175 and US 4,962,038.

25 Examples of compounds capable of minimizing bacterial growth are: 1,3-dimethylolurea and chlorhexidine diacetate as described in e.g. US 4,962,038.

Examples of compounds added to convert the hemoglobin species to an end-product suitable for spectrophotometric analysis are: potassium cyanide as described in e.g. US 4,485,175; US 4,745,071; US 4,528,274 and tetrazole or triazole as described in  
30 WO 99/49319.

Examples of particles or compounds which may be added in order to introduce a tool for minimizing variation between different batches of the disposable device are: latex beads of known size(distribution) and glass beads of known size(distribution).

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5 Addition E: If assisted mixing is required the mixing chamber might optionally include a mixing flea or a baffle. A magnetic flea may be used to force the convection through an externally moving magnetic field. A baffle may be used to mechanically stir the liquid when moved by an externally connecting mechanical device. This could be required if mixing with bubbles, such as bubbles blown into the sample through the aperture, is no possible.

Addition F: Multiple volume metering arrangements can be successively included if the test must deal with different concentrations of the different particles.

10 Addition G: A lid or coating tape may be used to cover the sample inlet before use. This ensures a clean sampling area at the origination of the test.

Addition H: A waste chamber may be applied at the outlet of the volume metering cell for waste or overflow of liquid.

Addition I: At any connection ports, e.g. the connection port to the pressure source, a small valve can be integrated to prevent liquid to leak out of the cartridge.

15 Addition J: A piston or membrane can be integrated into the cartridge to include a source of pressure for moving the liquid. The piston or membrane could be moved by a mechanical force provided by the instrument.

20 Addition K: An optical window can be integrated into the cartridge in order to perform optical measurements such as spectrophotometric detection of the hemoglobin content in a blood sample.

The methods described can be combined to give the best solution for the final application. The disposable sensor is particularly usable where portable, cheap, simple or flexible equipment is needed, such as in small laboratories, in measurements in the field or as a "point of care" ("near-patient") diagnostic tool.

25 When using the Coulter principle the diluent for use in the apparatus according to the invention may contain inorganic salts rendering the liquid a high electrical conductivity. When sample is applied to the electrolyte, the electrolyte to sample volumes should preferably be higher than 10. Sample preparation should preferably result in between 1.000 to 10.000.000 particles per ml and more preferably between  
30 10.000 and 100.000 particles per ml. A mixing of the sample after adding electrolyte is recommended. Particle diameters should preferably be within 5 to 50 percent of the orifice diameter and more preferably between 10 to 30 percent of the orifice diameter. Volume flow should preferably be from 10  $\mu$ l to 10 ml per minute and more preferably

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between 100  $\mu$ l and 1 ml per minute. For the measurement a constant electrical current of approximately 1 to 5 mA should preferably be applied. The source of electrical current should preferably have a signal to noise ratio (S/N) better than 1.000. The response from the electrode can be filtered electronically by a band-pass filter. A second order Chebyscheff filter with center frequencies around 1-10 kHz is suggested.

The invention will be further described and illustrated with reference to the accompanying drawings in which:

Fig. 1 shows a cross sectional side view through the components of a disposable unit 85, referred to as the cartridge,

Fig. 2 shows the flow-through sensor concept

Fig. 3 shows an apparatus based on the disposable cartridge, a docking station 66 and a reader 74,

Fig. 4 shows the cartridge with a build in piston,

Fig. 5 schematically illustrates the sampling procedure,

Fig. 6 is a plot of results obtained in Example 1,

Fig. 7 is a plot of results obtained in Example 2,

Fig. 8 is a plot of results obtained in Example 3,

Fig. 9 is a plot of results obtained in Example 4,

Fig. 10 is a plot of results obtained in Example 5,

Fig. 11 is a schematic illustration of the cartridge and hydraulic connections in example 6,

Fig. 12 is a histogram with all blood cells present in example 7,

Fig. 13 is a histogram of a result of removing the red blood cells in example 8,

Fig. 14 is a plot of the process described in example 9,

Fig. 15 is a plot of the process described in example 10,

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**DESCRIPTION OF PREFERRED EMBODIMENTS****Figure 1**

A disposable unit for blood analysis comprises a liquid storage chamber (1) containing a liquid diluent (11) a collecting unit (2) where blood (8) when applied will be filled into a capillary tube (10) by capillary forces and by switching said sampling unit (2) to a second position to connect said capillary tube (10) with said storage chamber (1) and said liquid diluent (11) to enable a dilution of said blood (8), hereafter the mixed blood (8) and diluent (11) are transported into a mixing chamber (3) and from the mixing chamber through a flow-through sensor (4) into a back chamber (5). A volume metering arrangement is connected to the back chamber comprising a volume metering chamber (6) having the size of the volume to be measured during the measurement with two connecting channels (12 & 13) of relatively diminutive internal volumes for registering liquid entry and exit by optical or electrical means, from the volume metering chamber a channel (7) leads out to a connection port 67 where a pressure can be applied.

**Figure 2**

The flow-through sensor (4) has a dividing wall (58) with a relatively thin passage (59) for the passage of particles suspended in liquid. The passage serves as a sensing zone for detection and measurement of the individual cells. The passage in the sensor may be formed as a count orifice for counting and sizing particles by an impedance method known as Coulter counting. Particles can be aspirated through the orifice by pressure driven flow in either direction. When a saline or other electrolytic liquid solution is added to the chambers, the two chambers will be electrically isolated from each other except for the route for current flow provided by the passage through the orifice.

**Figure 3**

The chambers on each side of the flow through sensor may have electrodes 34, 35 extending from an external terminal 61, 62 through the base wall 63 of the disposable unit and into a configuration facing the inside of its respective chamber. The cartridge is placed in a docking station 66 in a portable apparatus in order to carry out the test. The docking station 66 has a cup shaped housing having a base 70 and a circumambient side wall 71. In the base 70 there are respective spring loaded electrical connectors 64, 65 for contacting the terminals 61, 62 of the cartridge



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automatically when the cartridge is received as a push fit into the docking station. There is also a conduit 68 passing through the base wall 70 aligned with the conduit 67 of the cartridge. Conduit 67 at its opening into the upper face of the wall 70 has a seal 69, such as e.g. an O-ring for forming a gas tight connection with the lower face of the base wall 63 of the cartridge. A vacuum pump 72 is connected by a line 73 to the lower end of the conduit 68. In a modification of the apparatus, the vacuum pump 72 can be reversed so as to apply positive gas pressure to the conduit 68. Schematically indicated at 74 are the further conventional components of a Coulter counter including all the electronic circuitry and display equipment needed for the operation of the apparatus.

#### Figure 4

As an alternative to the gas pump a piston 9 could be built into the cartridge for directly applying a negative or positive pressure.

#### Figure 5

Fig. 5 schematically illustrates the blood sampling operation. The illustrated part of the cartridge 2 includes the liquid storage chamber 83 for storing a diluent for diluting the sample and the mixing chamber 77 for mixing the sample 84 and the diluent. This figure schematically illustrates a device for sampling a small and accurate volume of liquid in accordance with the present invention. The device 10 comprises a first member 86 with a first opening 87 for entrance of a liquid sample into a first cavity 75 in the first member 86 and with a second opening 76 for outputting the liquid sample from the first cavity 75. The first cavity 75 forms a capillary tunnel. The first opening 87 of the first member 86 may be brought into contact with a liquid 8 (shown in Fig. 1), 84 to be sampled so that the liquid 84 may flow through the first opening 87 into the first cavity 75 and out of the second opening 76 by capillary attraction. The device 12 further comprises a sampling member 78 with a second cavity 82 for receiving and holding the liquid sample 84 and having a third opening 88 communicating with the second cavity 82. The second cavity forms a capillary tunnel with essentially the same diameter as the first cavity 75. The sampling member 78 is a circular cylinder that is movably positioned in relation to the first member 86. During sampling of the liquid, the sampling member 78 is positioned in the illustrated first position in relation to the first member 86 wherein the second opening 76 is in communication with the third opening 88 so that sampled liquid may flow through the second 76 and third opening 88 into the second cavity 82 by capillary attraction. The third opening 88 may

be disconnected from the second opening 76 in a second position of the sampling member 78 in relation to the first member 86 so that the liquid sample 84 contained in the second cavity 82 is disconnected from the first cavity 75.

5 The sampling member 78 is inserted into a third cavity 34 of the first member 86 for receiving and accommodating a part of the sampling member 78. The sampling member 78 may be displaced between the first and second position along a longitudinal axis of the sampling member 78 that is also substantially perpendicular to a longitudinal axis of the second cavity 82. The sampling member 78 may also be  
10 rotatable about a longitudinal axis that is substantially perpendicular to a longitudinal axis of the second cavity 82. In the first position, the first 75 and second 82 capillary tunnels extend along substantially the same longitudinal center axis.

15 In the illustrated embodiment the first member 86 is symmetrical and has a fourth cavity 80 with openings 81, 79 opposite the first cavity 75, and the sampling member 78 has an opening 89 opposite the opening 88 so that, in the first position, a capillary tunnel extends through the first 86 and the second 78 member and communicates with the environment through openings 87, 79. Thus, air may escape from the capillary tunnel through opening 79. Further, in the first position, a part of the liquid entering the second cavity 82 will leave the cavity 82 through opening 89 thereby  
20 ensuring that the cavity 82 has been completely filled with liquid during liquid sampling eliminating the risk of sampling with a reduced sample volume leading to low accuracy sampling.

25 Fig. 5a illustrates the device 2 ready for receiving the liquid. In Fig. 5b, a sample has entered into the capillary tunnel 82, and in Fig. 5c the sampling member 78 has been rotated into the second position for isolation of an accurate volume of the sample 84, and finally Fig. 5d illustrates that the sample 84 has been washed out of the capillary tunnel 82 and into the mixing chamber 77 by the diluent.

Example: The capillary tunnel forming the second cavity 82 may have a length of 8 mm and a diameter of 0.9 mm for containing a liquid sample of 5.089  $\mu\text{L}$ .

30 Example: The capillary tunnel forming the second cavity 82 may have a length of 5 mm and a diameter of 0.5 mm for containing a liquid sample of 0.982  $\mu\text{L}$ .

Example: The capillary tunnel forming the second cavity 82 may have a length of 3 mm and a diameter of 0.3 mm for containing a liquid sample of 0.212  $\mu\text{L}$ .

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Figure 6**Example 1 – Sizing of polymer beads**

A mixture of 5  $\mu\text{m}$  and 10  $\mu\text{m}$  particles suspended in electrolyte was aspirated through the orifice of the apparatus shown in Fig. 3. The numbers of particles  
5 detected and the size of each detected particle were recorded. A bimodal distribution of detected particle size is clearly seen in Fig. 6.

Figure 7**Example 2 – Red blood cell counting**

Measurement of blood cells has been performed and the result is shown in Fig. 7.  
10 Red blood cells are normally around 5 to 7  $\mu\text{m}$  in diameter and are the most frequent in whole blood, as can be seen on the Fig. 7. The distribution is a Gaussian curve, as it should be expected. Blood counts can be used in clinical diagnostics. It is fairly simple to count erythrocytes, leukocytes and thrombocytes by impedance measurements, which is considered the basic parameters for hematology (see  
15 "Fundamentals of Clinical Hematology", Stevens, W.B. Saunders Company, ISBN 0-7216-4177-6).

Figure 8

**Example 3 – White cell counting using a diluent containing a reagent-composition selected so as to preserve all blood cells.**

**20 MATERIAL**

Cartridge and apparatus containing the functions as described in the present invention,

Isoton, Beckman Coulter (prod.no. 24655) containing: sodium chloride 7.9 g/L, potassium chloride 0.4 g/L, disodiumhydrogenphosphate 1.9 g/l,  
25 sodiumdihydrogenphosphate 0.2 g/L, disodium-EDTA 0.4 g/L and sodium fluoride 0.3 g/L.

Vacutainer, K<sub>3</sub>E, Becton & Dickinson, prod.no. 367652.

Bayer, ADVIA-120 equipment.

**PERFORMANCE**

30 The full sequence of the procedure was as follows:

- 18 -

- Collection of a venous blood sample in a vacutainer tube.
- Leaving the sample, for the sedimentation process to proceed, for three hours.
- Extraction the plasma phase with the major part of the buffy-coat section included
- Performing analysis using the Bayer Advia 120 equipment for obtaining a comparative value for the content of leukocytes.
- Adding 5.00 ml isoton solution as diluent to the chamber of the test rig
- Adding 10.0 µl of sample to the chamber
- Mixing liquids in the chamber
- Starting test sequence on the computer (starts the pump and readies the sampling)
- When the liquid reaches the first level electrode sampling is started
- When the liquid reaches the second level electrode the sampling is stopped
- Sampled values are saved in a file
- The file is opened with a "pulse-viewer" for data analyzing and calculation of the result using a method of calculation involving subtraction of, with the leukocytes, overlapping red blood cells.

**RESULTS**

20 Bayer Advia-120:  $11.96 \times 10^9$  leukocytes/L  
 Test-rig:  $11.92 \times 10^9$  leukocytes/L  
 Difference in accuracy:  $(11.96 - 1.92) / 11.96 = 0.33 \%$

**Figure 9**

25 Example 4 – White cell isolation using a diluent containing a reagent composition selected so as to primarily hemolyse the red blood cells.

**MATERIAL**

Cartridge and apparatus containing the functions as described in the present invention,

Diluent containing: procaine hydrochloride 0.10 g/L, 1,3-dimethylolurea 0.90 g/L, N-(1-acetamido)iminodiacetic acid 1.28 g/L, dodecyltrimethyl ammonium chloride 7.51 g/L and sodium chloride 0.03 g/L.

Vacutainer, K<sub>3</sub>EDTA, Becton & Dickinson, prod.no. 367652.

## 5 PERFORMANCE

The full sequence of the procedure was as follows:

- Collection of a venous blood sample in a vacutainer tube.
- Leaving the sample, for the sedimentation process to proceed, for three hours.
- Extraction the plasma phase with the major part of the buffy-coat section included
- Adding 2.000 ml diluent as described above to the chamber of the test rig
- Adding 4.0 µl of sample to the chamber
- Mixing liquids in the chamber
- Starting test sequence on the computer (starts the pump and readies the sampling)
- When the liquid reaches the first level electrode sampling is started
- When the liquid reaches the second level electrode the sampling is stopped
- Sampled values are saved in a file
- The file is opened with a "pulse-viewer" for data analyzing and generation of the result.

## RESULTS

As can be seen in the histogram in fig. 6 the particle population corresponding to the leukocytes are easily identified in the absence of the red blood cells.

### Figure 10

#### Example 5 – Counting somatic cells

Milk quality is essential for farmers, dairy producers and consumers. Farmer has to deliver milk of a certain quality which is controlled by the so-called Somatic Cell

- 20 -

- Count (SCC). In milk quality tests somatic cells in the milk are counted to determine infections (clinical mastitis). A limit of 400,000 cells pr. ml. has to be met by the farmers for dairy resale. Change of diet, stress or mastitis lead to higher SCC levels, thus lowering the quality of the milk and consequently lowering the price per unit volume. A cheap cell counter will help farmers and dairy producers monitor SCC-level.

#### Figure 11

##### Example 6 - A Blood Diagnostic System

- This is an example of a 3 part differential white blood cell count (monocytes, lymphocytes, granulocytes), thrombocytes count and hemoglobin measurement and the corresponding instrumentation and cartridge realized through the present invention.

A three part differentiation of white blood cells, thrombocyte counter with measurement of hemoglobin can be achieved with the specified components.

- A reagent for selectively lysing red blood cells is added to the diluent in the storage chamber (1). When the whole blood (8) is added to the opening (58) of the first capillary section (15), the blood will be dragged in to the capillary and through the middle section (10) and last section (14) of the capillary. The last section of the capillary is connected to a fill-chamber (43) for visually verification of the filling. The fill-chamber (43) is connected through a conduct (44) to open air.

- The blood filled middle section of the capillary is part of a knob (2) that can be moved to a second position, connecting the ends of the capillary to two other conducts, a conduct (45) connected to the storage chamber (1) and a second conduct (40) connected to the mixing chamber (3) respectively. A third conduct (39) is leading from the mixing chamber to a port opening (42) in the cartridge. The port opening is connected through a counter port opening (37) in the apparatus, through a tubing (46) to a three position valve (51) and directed through the two positions of the valve to open air through a second tubing (55) or through a third tubing (50) to the suction port of a membrane pump (47).

- When the blood and diluent with reagent has been sucked into the mixing chamber, the blood can be mixed by blowing bubbles through the aperture of the sensor (4). The air pressure is applied through the back chamber (5), via a fourth conduct (12A), a small volume chamber (6A), a fifth conduct (12B), a large volume chamber (6B)

and a sixth conduct (7) directed to an opening port (41) in the cartridge. A counter port (36) in the apparatus is connected through a fourth tubing (48) to a second three position valve (52), which has positions to direct to both vacuum through a fifth tubing (56) to the suction port of the membrane pump, or to the exhaust of the membrane pump, through a third two position valve (53) and a sixth tubing (49), said third valve having two positions for said connection and for directing the pump exhaust to open air through a seventh tubing (54) respectively.

After mixing the diluted and lysed blood (red blood cells is removed) it is ready to be measured. The mixing chamber is connected through the first valve to open air and the back chamber is connected through the second valve to the suction port of the pump. The exhaust of the membrane pump is connected through the third valve to open air. As the blood and diluent flows from the mixing chamber into the back chamber, an electrical connection between to counter electrodes (34) and (35) placed in each chamber is established through the liquid. Cells are counted and differentiated by size by the Coulter principle. Through sizing of the cells, the cells can be parted into different groups containing cells of a certain type. Thus white blood cells (leucocytes) can be differentiated into granulocytes, lymphocytes and monocytes. Furthermore, thrombocytes (platelets) can be differentiated from leucocytes as well. In order to determine the concentration, the volume of the diluted blood which has been counted must be known. Since thrombocytes are approximately ten times as frequent as leucocytes, it may be necessary measure two different volumes. The thrombocytes are counted according to a small volume (6A) which placed between the back chamber and the larger volume. By registering the liquid entry and exit at the inlet and outlet of the small volume chamber respectively, the counting period will be given. Registration of the liquid level is preferably done by an optical reflectance measurement at the inlet (33) and at the outlet (32). The outlet of the small volume chamber is also the inlet of the large volume chamber (6B). This chamber is used for counting of leucocytes. At the outlet of the large volume chamber, a third optical reflectance measurement (31) is performed to register the exit of the liquid from this chamber.

After counting both leucocytes and thrombocytes the hemoglobin content can be measured by optical spectroscopy preferably through the middle section of the large volume chamber (30).

Process of the test (example 6):

The process of making a test by means of the present invention can be characterized as:

- 1) Draw blood by using a lancet device
- 2) Pick up blood droplet by touching the blood to the cartridge inlet
- 5 3) Mount cartridge in the instrument (instrument starts and runs the test)
- 4) Read the result from the display
- 5) Remove and waste cartridge

Figure 12

Example 7 Waveguides for optical detection of cells

- 10 It is possible to measure structural as well as functional cell parameters with an optical measurement method, since fluorescent labeled reagents or probes can be applied in order to stain specific parts of the cells. Presently, several thousand probes and reagents are available for staining specific parts of the cell such as the cell membrane, DNA, antibodies, protein, etc.
- 15 Y. Fu et al, "A micro fabricated fluorescence-activated cell sorter", Nature Biotechnology, Vol. 17, November 1999, pp.1109-1111 and Bernhard H. Weigl, "Micro fluidics-based lab-on-a-chip", IVD Technology Magazine, Nov-Dec 2000 disclose micro machined optical flowcytometers which are intended to provide cheap, portable blood analyzers. However, these instruments still require precision fluidic
- 20 control, a laser source and a sensitive optical detection system. Such components may prove hard to miniaturize into a portable system.

In WO 01/69202, which is hereby incorporated by reference, a sensor is disclosed for characterizing particles suspended in a liquid and having at least two optical waveguides for optical characterization of particles passing an orifice.

- 25 Thus, the particle characterization means may include a first waveguide positioned for optical communication with a particle located in the orifice, and may further include a second waveguide also positioned for optical communication with a particle located in the orifice.

- 30 The waveguides may be built into the wall containing the orifice so that each of the waveguides are kept in optical communication with a common location in the orifice, the location constituting a measurement or sensing location.



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Preferably, the length of the orifice or passageway is from 1 to 100  $\mu\text{m}$ , for example about 50  $\mu\text{m}$ . Desirably the axial length of the passageway is chosen such that only one particle will be present in the passageway at the time when detecting particles of from 0.1 to 100  $\mu\text{m}$  diameter. The aspect ratio of said passageway, (axial length divided by diameter) is preferably from 0.5:1 to 5:1, more preferably from 1:1 to 3:1.

Preferably, the largest cross-sectional dimension of said passageway at said common location is from 5 to 200  $\mu\text{m}$ , for example 10 to 50  $\mu\text{m}$ .

The preferred transverse dimensions of the waveguides are related to the width of the passageway and to the diameter of the particles to be measured. Suitably, each said waveguide adjacent said common location has a maximum cross-sectional dimension of from 1 to 200  $\mu\text{m}$  more preferably 5 to 200  $\mu\text{m}$ , e.g. 5 to 50  $\mu\text{m}$ , but preferably, the waveguide width is similar to the width of the orifice so as to illuminate and receive from, respectively, the entire orifice through which particles may pass. The diameter of the waveguide (i.e. the transverse dimension in the axial direction of the passageway) is preferably similar to the diameter of the particles and preferably does not exceed this.

Preferably, the waveguides extend from said common location in said passageway radially from the passageway. They may be angularly separated from one another at said common location by intervening angles of for example 45°, 90°, 135° or 180°. Preferably, there are at least four waveguides which are angularly separated from one another by intervening angles of 90°. Adjacent waveguides can be used also in setting up the optimal alignment of the optical fiber or other optical interfacing at the edge of the sensor unit in that such an optical fiber or other optics can be moved with relation to its respective waveguide horizontally and vertically until a maximum signal is obtained through the adjacent waveguide.

The waveguides will comprise a core material of higher refractive index surrounded by a cladding of lower refractive index. Said core material and cladding material are preferably formed in situ by a process comprising deposition of layers of material, photolithographic masking and etching.

This may comprise forming a first layer of cladding material, optionally on a substrate, forming over this layer of core material, masking waveguide tracks in said core material and etching away non-masked areas, de-masking the remaining core

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material and forming a second layer of cladding material between and over said core material.

Alternatively, the core material may be formed from the cladding material by exposing the cladding material to light such as locally to alter the refractive index of the cladding material.

6

When operating in this manner to form the waveguides the cladding material is preferably a Germanium doped silica.

When practicing the method of layer deposition or the method of refractive index alteration, the wall or membrane is preferably constructed by depositing a glass or a glass-like material on the surface of a silicon wafer. Glass or glasslike materials that are suitable include silicon-compound glasses, e.g. doped or non-doped silica glass, silicon nitrate and silicon dioxide. other possible substrates include GaAs, Kovar, Invar or other materials having a similar thermal expansion coefficient which should be almost identical to glass over the range of 0 to 4000 °C. After deposition of the glass layer(s), the silicon wafer may be selectively etched to form a membrane comprised of said glass or glass like material and a reduced thickness of the silicon layer or only of glass or glass like material.

10

15

More preferably, waveguides can be fabricated by deposition of a polymer such as polyamide that can be structured by lithographical means (or by other means such as micro machining and embossing) on a substrate. Requirements to the substrate are less restrictive, but the surface should be able to bind the polymer.

20

Thus, the particle characterization apparatus may comprise a cartridge as described, a light source optically coupled to a first of said waveguides and an optical detector optically coupled to a second of said waveguides. The detector may be for detecting adsorption, scattering or fluorescence.

25

The apparatus may further comprise a pump connected to pass a liquid sample containing suspended particles through said passageway. This may operate to feed liquid from upstream to said passageway or to aspirate liquid from downstream through said passageway.

30

There may also be a pump connected to pass a flow of sheath liquid through said manifold when this is present to form an annular sheath flow surrounding said liquid sample as the liquid sample passes through said membrane orifice, and a flow rate

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controller controlling the flow rate of said sheath flow through said orifice to variably restrict the diameter of the flow of liquid sample through said orifice.

As explained above, the present invention provides in preferred aspects a sensor based on a membrane essentially fabricated by micro machining. The membrane has an orifice placed relatively in the center of the membrane, which can be used for aspiration of particles suspended in a liquid, as the sensor is submerged into the liquid. This way of transporting particles into a measuring region is known for electrical characterization of particles by the Coulter principle (V. Kachel, "Electrical Resistance Pulse Sizing Coulter Sizing", Flow Cytometry and Sorting, 2. ed., pp 80, 1990 Wiley-Liss, Inc.). In order to illuminate the particle or detect light from the particles a number of light guiding structures can be placed on the surface of the membrane. Such light guiding structures can be formed when a material of relatively high transmission coefficient has a refractive index relatively higher than the surrounding material, (which also has relatively high transmission coefficient). These structures are also known as Waveguides (see K. Hoppe, M. Svalgaard, M. Kristensen, "Integrated Optical Waveguides in Fluidic Microsystems" Analytical methods and Instrumentation, Special Issue  $\mu$ TAS 196, side 164-166). Excitation and detection of the particles can both be performed with the waveguides. The formation of the waveguides aligned to the orifice can easily be established by the use of lithography or by laser writing the regions of higher refractive index directly on the material. The sensor can easily be coupled to optical fibers connected to the light source and detectors, and by placing tubes on either end of the sensor, particles can be transported through the orifice and detected without the use of precision optics and sheath fluid. In addition the measurements can also be combined with measurements of impedance for Coulter-sizing.

#### Figure 13

##### Example 8 Annular manifold for hydrodynamic focusing of the cell flow

The membrane may comprise an annular manifold within the thickness of the membrane, an outlet or series of outlets from said manifold annularly surrounding said orifice on one surface of said membrane, and a flow path within the thickness of the membrane communicating between an inlet to said flow path and said annular manifold. For this purpose, the membrane may comprise a silicon layer and a glass or glass like material layer overlying the silicon layer and containing waveguides as

discussed in example 7, and said manifold and flow path may lie between the silicon layer and the glass or glass-like material layer.

Thus, the cartridge may comprise a membrane having an orifice extending through the thickness of the membrane for the one at a time passage of particles suspended in a liquid, an annular manifold within the thickness of the membrane, an outlet or series of outlets from said manifold annularly surrounding said orifice on one surface of said membrane, and a flow path within the thickness of the membrane communicating between an inlet to said flow path and said annular manifold. As described above the membrane may comprise a silicon layer and a glass or glass like material layer overlying the silicon layer, and said manifold and flow path may lie between the silicon layer and the glass or glass-like material layer.

#### Figure 14

##### **Example 9 Photolithography**

An orifice may suitably be formed in a photo-reactive polymer by photo-lithography and subsequent development. Thus a free standing sheet of polymer of the kind used conventionally as a photo resist material may be exposed to light to render a spot to soluble to define an orifice (or to render the non-spot forming areas in-soluble) followed by development with solvent to remove material to form the orifice. Normally, a large number of count wafers each containing a respective orifice will be made simultaneously in one sheet. Suitable photo resist polymers are described in e.g. M. Madou "Fundamentals of Micro fabrication, CRC Press LLC, 1997, ISBN 0-8493-9451-1. They include AZ-5214E, SU8, polyamides and others.

Alternatively, the photo resist polymer may be used as a protecting layer over a substrate such as silicon in which the orifice is formed by etching regions exposed by development of the photo resist. If the etched substrate is electrically conducting it may be insulated prior to use by the formation of a suitable insulating layer there over. The photo resist polymer may be used as such a layer.

Count wafers made lithographically may be used in all forms of apparatus and method according to this invention. Figure 14 shows one process of fabricating the count wafer: (a) appliance of a thin sheet of photo resist. (b) Development of the mask. (c) Etching of the aperture by Deep Reactive Ion Etching (DRIE, M. Madou "Fundamentals of Micro fabrication, CRC Press LLC, 1997, ISBN 0-8493-9451-1).

#### Figure 15

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**Example 10 Aperture fabricated by laser micro machining**

Apertures for Coulter counting can be fabricated by laser micro machining of polymers, which could lead to a simple and convenient way of fabricating and assembling apertures for the cartridge. A series of small holes of 50  $\mu\text{m}$  has been  
5 fabricated with an UV-laser. The holes are made in less than 1ms in a 50  $\mu\text{m}$  polymer sheet. The uniformity of the holes is very high and the smoothness of the aperture entrance is unique. Figure 15 shows the process of laser machining of the aperture. The laser cuts through the polymer foil in a circle, thus defining the size of the aperture.

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## CLAIMS

1. A cartridge for characterizing particles suspended in a liquid, comprising a housing with
- 5 a mixing chamber and a collection chamber separated by a wall containing an orifice for the passage of the particles between the mixing chamber and the collection chamber,
- particle characterization means for characterizing particles passing through the orifice,
- 10 a first opening in the outer surface of the housing for entrance of a liquid sample into a first cavity in the housing and with a second opening in the housing for outputting the liquid sample from the first cavity, and
- a sampling member positioned in the housing for sampling a small and precise volume of liquid and having a second cavity for receiving and holding a liquid sample and having a third opening communicating with the second cavity, the member being movably positioned in relation to the housing in such a way that,
- 15 in a first position, the second opening is in communication with the third opening for entrance of the liquid sample into the second cavity, and, in a second position, the third opening is in communication with an inlet to the mixing chamber.
- 20 2. A cartridge according to claim 1, wherein the particle characterization means includes a first electrode in the mixing chamber and a second electrode in the collection chamber, each electrode being electrically connected to a respective terminal member accessible at the outer surface of the cartridge.
3. A cartridge according to claim 1 or 2, wherein the particle characterization means
- 25 includes a first wave guide positioned for optical communication with a particle located in the orifice.
4. A cartridge according to claim 3, wherein the particle characterization means includes a second wave guide positioned for optical communication with a particle located in the orifice.

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- 5 5. A cartridge according to any of the preceding claims, wherein the housing further comprises a liquid storage chamber for holding a liquid and having an outlet that in the second position of the sampling member communicates with a fourth opening in the sampling member communicating with the second cavity so that liquid can be discharged from the liquid storage chamber through the second cavity of the sampling member and into the mixing chamber together with the liquid sample when the sampling member is in its second position.
- 10 6. A cartridge according to any of the preceding claims, comprising volume metering means for determining the beginning and end of a period during which a predetermined volume of liquid has passed through the orifice.
7. A cartridge according to claim 6, wherein the volume metering means comprises a volume metering chamber with an input communicating with the collection chamber and an output, and wherein presence of liquid is detected at the input and at the output, respectively.
- 15 8. A cartridge according to claim 7, wherein presence of liquid is detected with a secondary electrode positioned at the input and a further secondary electrode positioned at the output.
9. A cartridge according to claim 7, wherein presence of liquid is detected optically.
- 20 10. A cartridge according to any of the preceding claims, wherein each of the mixing chamber and the collection chamber has a transverse cross-sectional area at the level of the orifice which is substantially less than the transverse cross-sectional area of the respective chamber over a substantial part of the height of the chamber above the orifice.
- 25 11. A cartridge according to any of the preceding claims, wherein the surface defining the second cavity of the sampling member has an anti-coagulation reagent.
12. A cartridge according to any of the preceding claims, wherein the storage chamber holds chemical reagents for modification of the blood sample.
- 30 13. A cartridge according to any of the preceding claims, wherein a mixing member is positioned in the mixing chamber.
14. A cartridge according to claim 13, wherein the mixing member is magnetic.

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15. A cartridge according to any of the preceding claims, further comprising a sensor for characterization of the liquid.
16. A cartridge according to claim 14, wherein the sensor for characterization of the liquid is adapted for spectro-photometric characterization of the liquid.
- 5 17. A cartridge according to any of the preceding claims, wherein the housing further comprises a pump chamber communicating with the collection chamber and having a pump actuator for causing a liquid flow through the orifice.
18. A cartridge according to claim 17, wherein the pump actuator is a piston.
19. A cartridge according to claim 17, wherein the pump actuator is a membrane.
- 10 20. A method of operating a particle characterization apparatus comprising a cartridge according to any of the preceding claims, the cartridge being demountable from the apparatus, the method comprising
- 15 sampling liquid containing particles with the cartridge through the first opening with the sampling member in its first position,
- positioning the cartridge in the apparatus,
- moving the sampling member to its second position,
- pumping liquid in the storage chamber through the second cavity and into the mixing chamber together with the liquid sample,
- making particle characterizing measurements,
- 20 disconnecting the cartridge from the apparatus, and
- discarding the cartridge.
21. An apparatus for characterizing particles suspended in a liquid, comprising a cartridge according to any of claims 1-19, and a docking station for removably receiving the cartridge,
- 25 the docking station comprising
- connectors for operational connection with the particle characterization means when the cartridge is received in the docking station.

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22. An apparatus for characterizing particles suspended in a liquid, comprising a cartridge according to any of claims 1-16, and a docking station for removably receiving the cartridge,

5 the cartridge further comprising a port communicating with the collection chamber for causing a liquid flow through the orifice,

the docking station comprising

connectors for operational connection with the particle characterization means when the cartridge is received in the docking station, and

10 a port for forming a gas connection with the with the cartridge port when the cartridge is received in the docking station for application of a pressure causing a liquid flow through the orifice.

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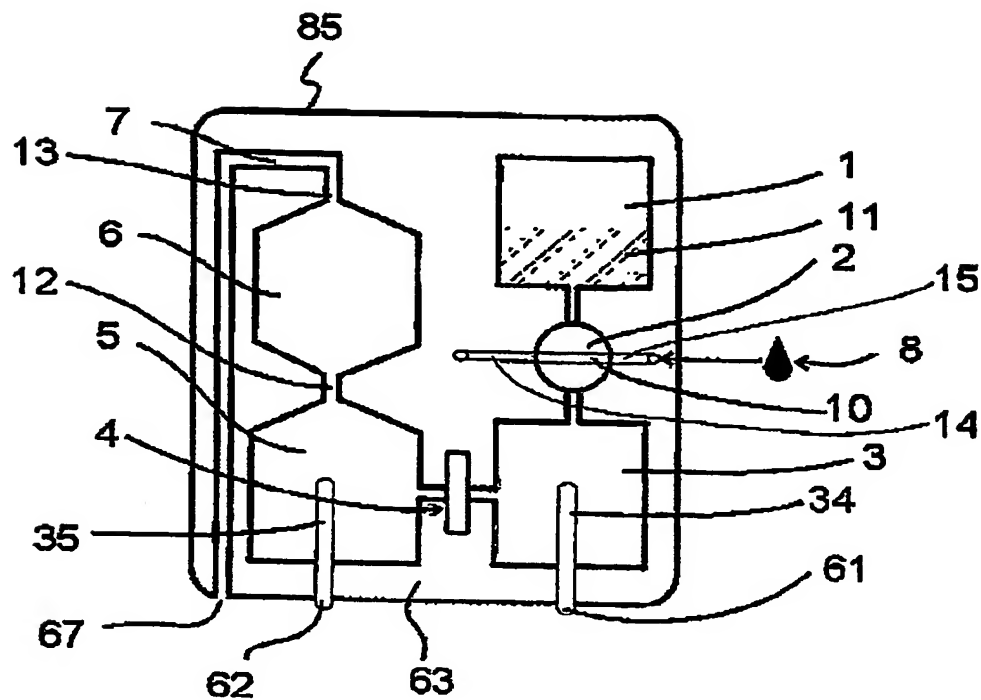


Fig. 1

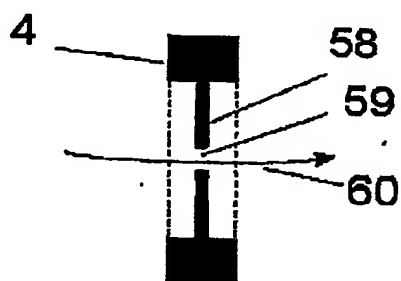


Fig. 2

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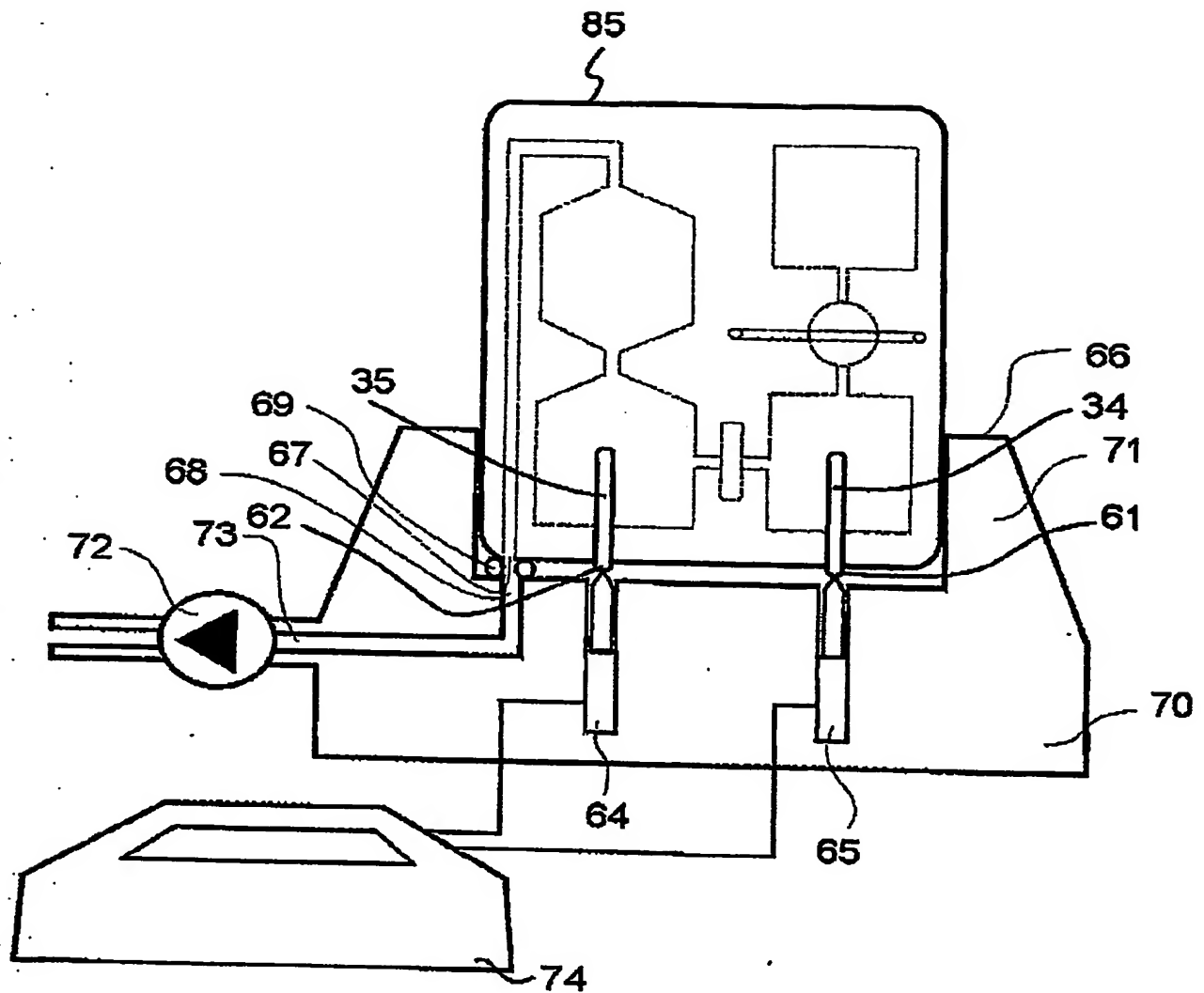


Fig. 3

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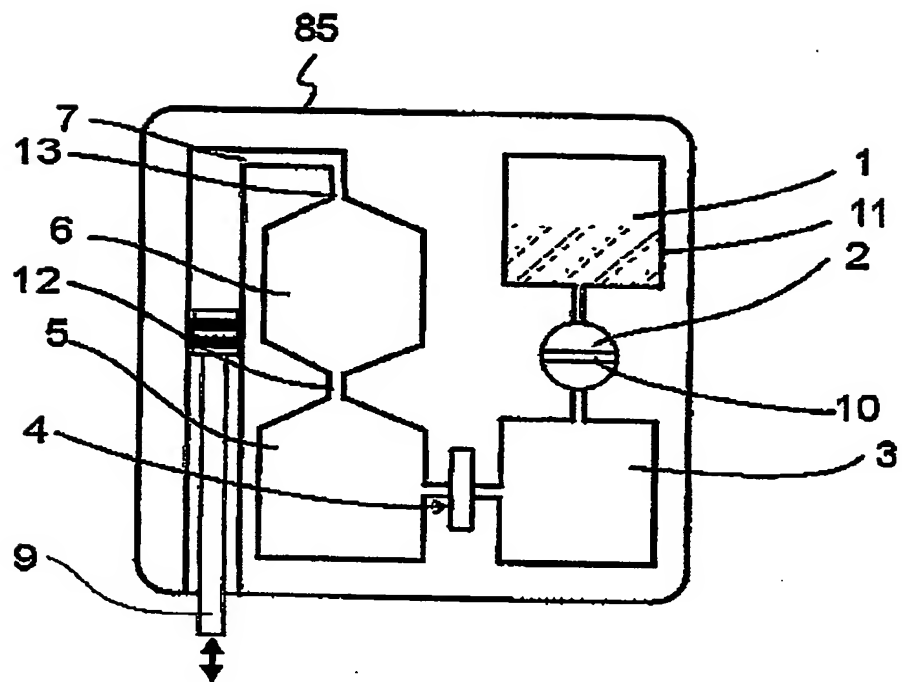


Fig. 4

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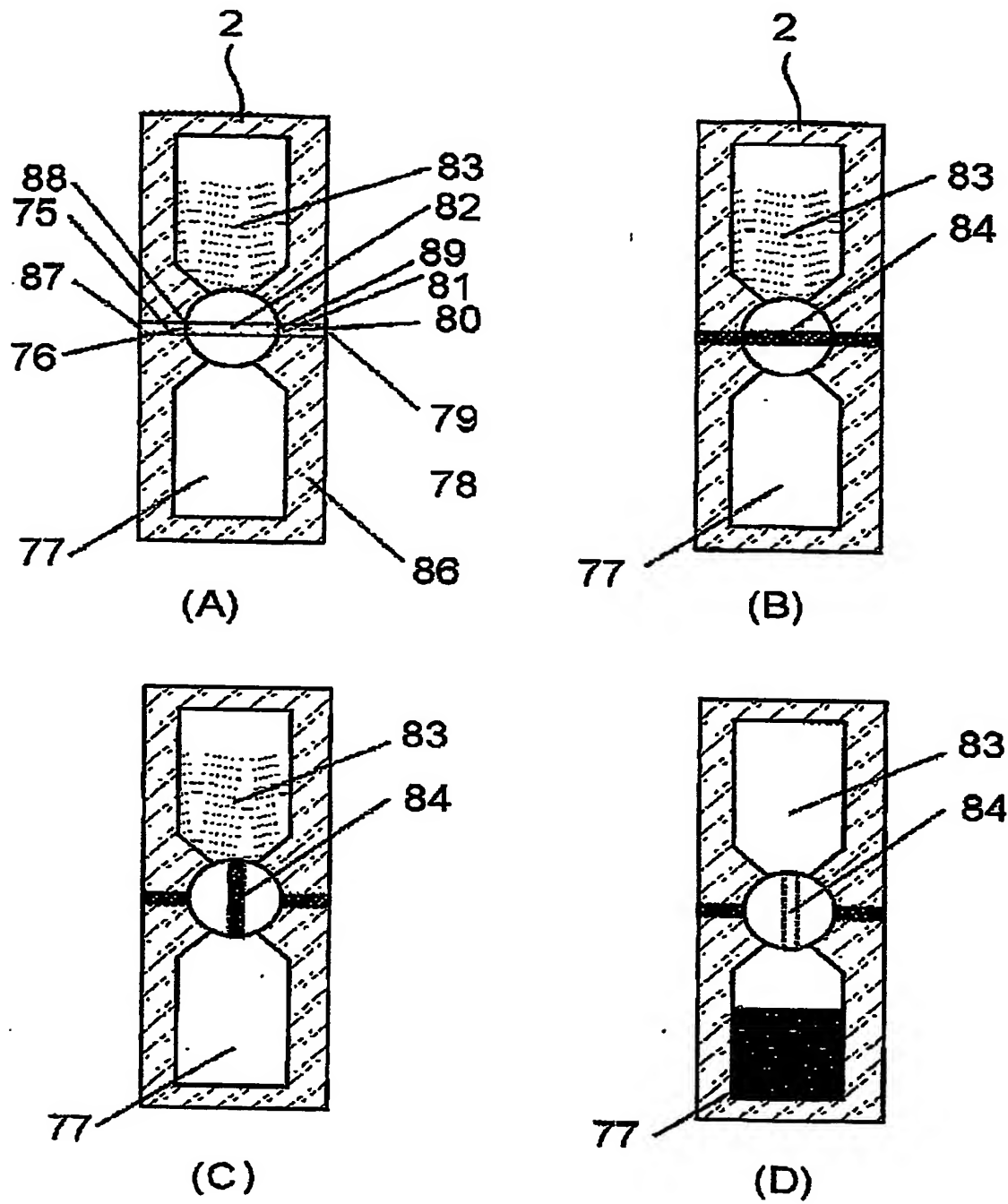


Fig. 5

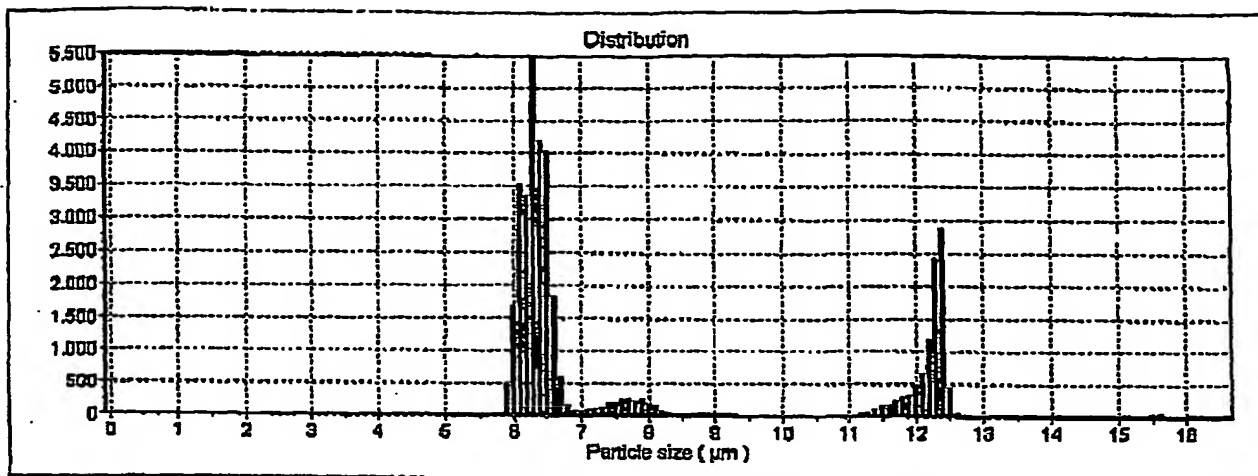


Fig. 6

### Blood cell characteristic

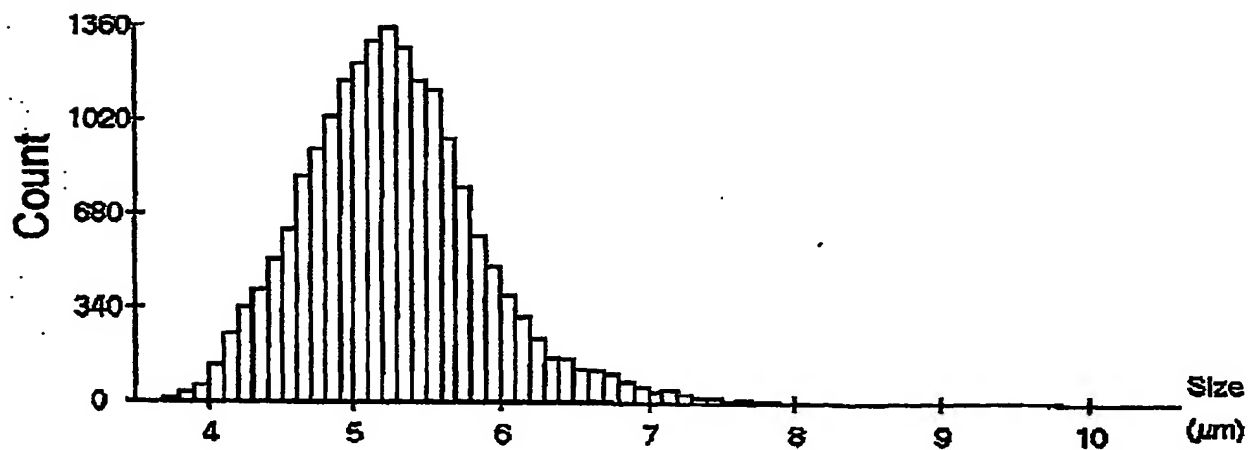


Fig. 7

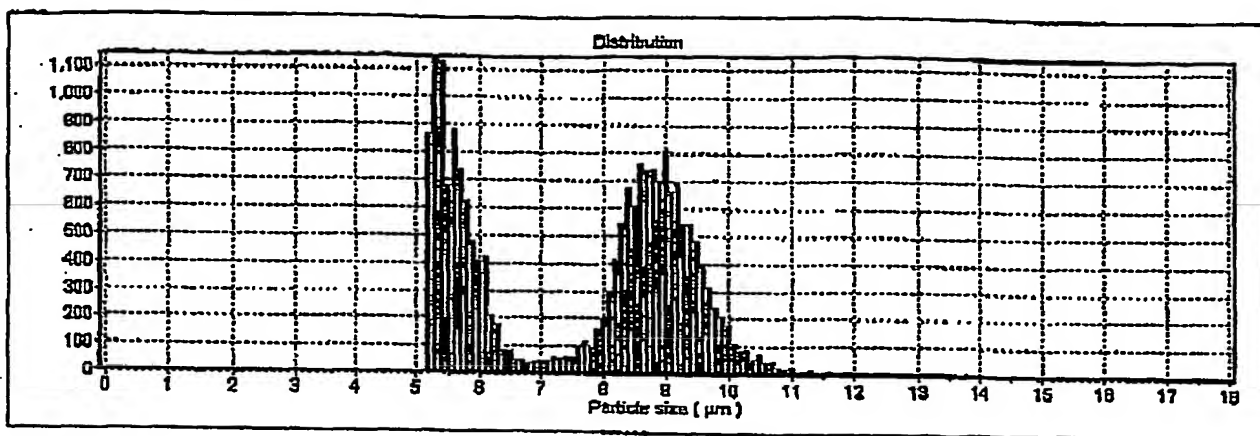


Fig. 8

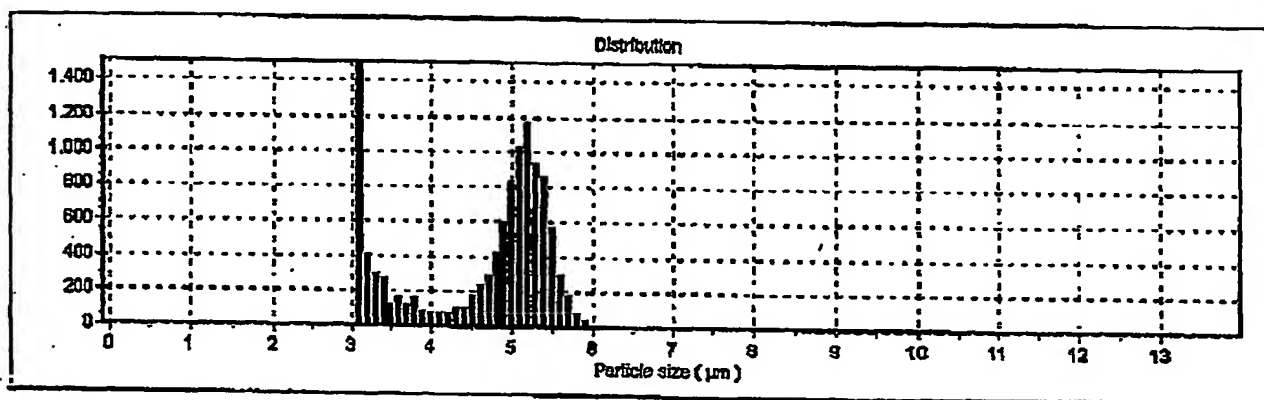
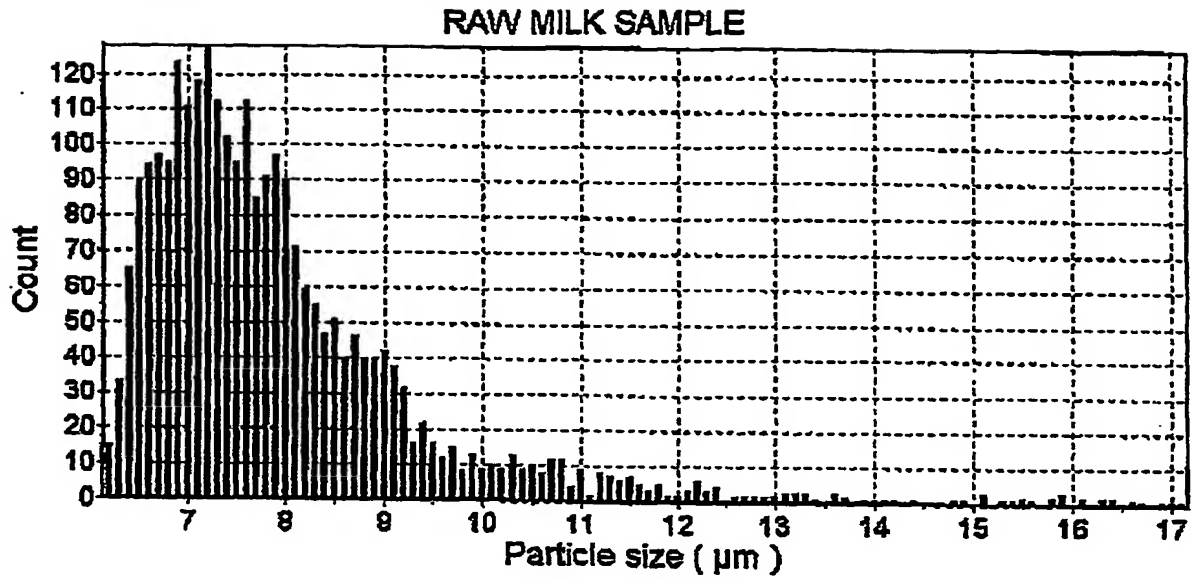


Fig. 9

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**Fig. 10**

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50387407.061102

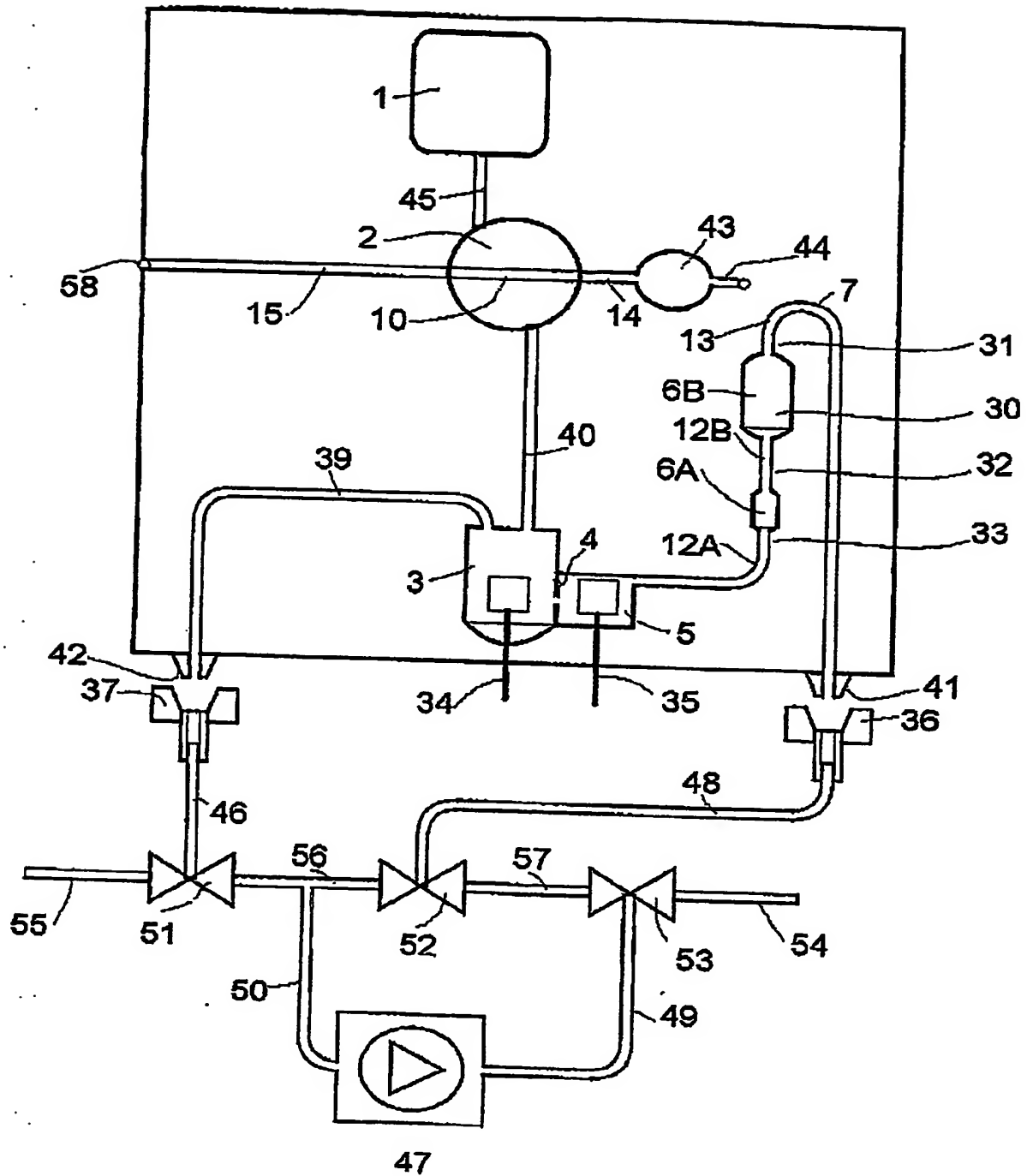


Fig. 11

60387407-061102

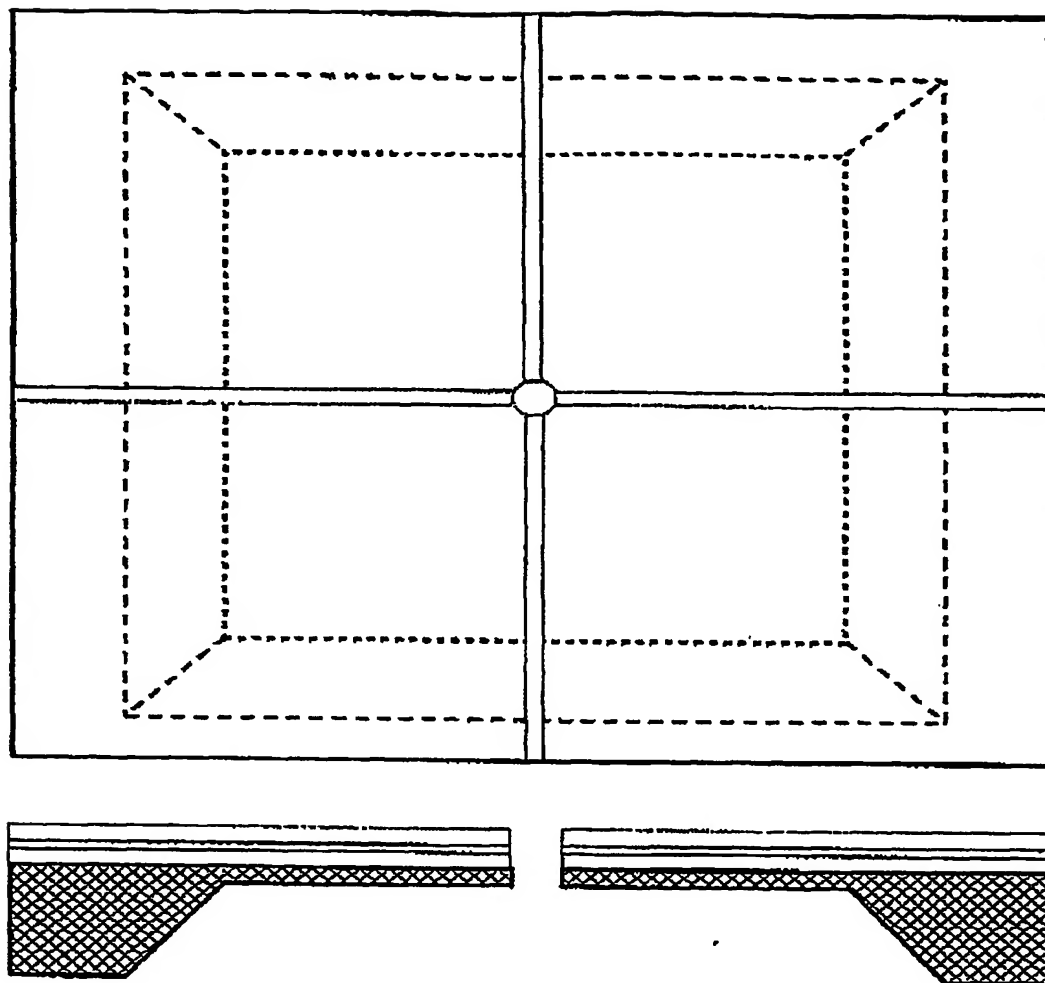


Fig. 12

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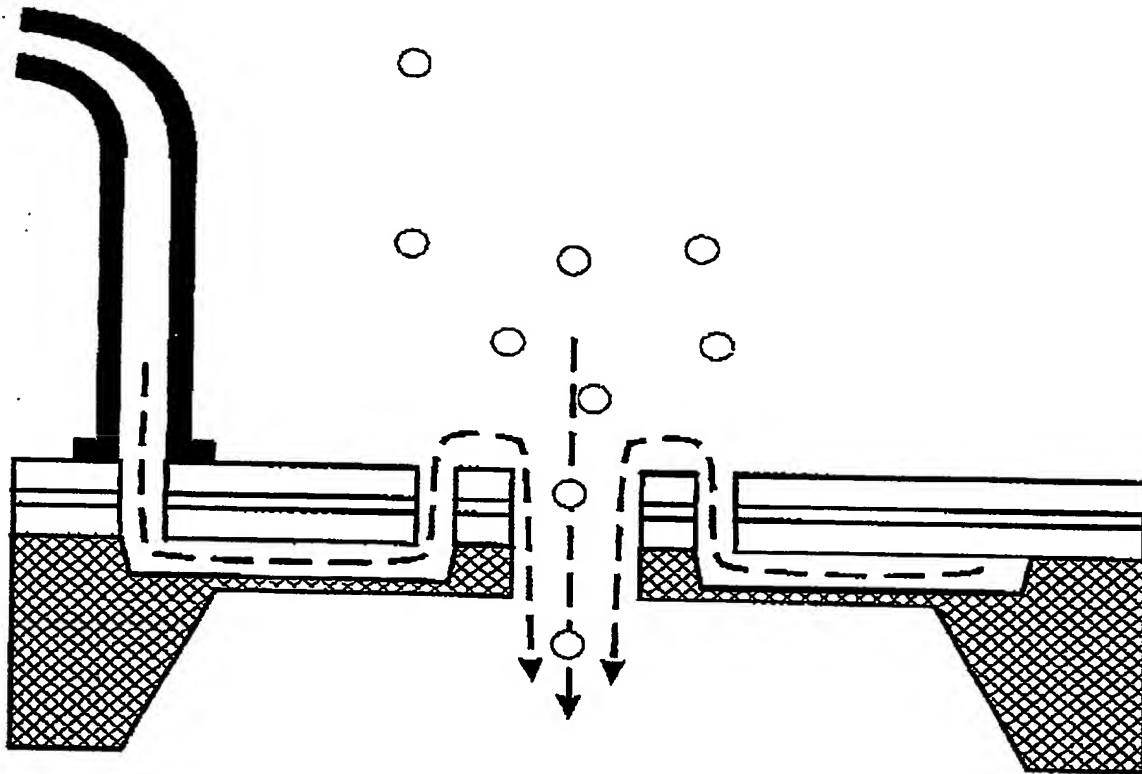


Fig. 13

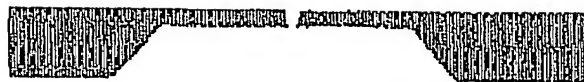
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(A)



(B)



(C)

Fig. 14

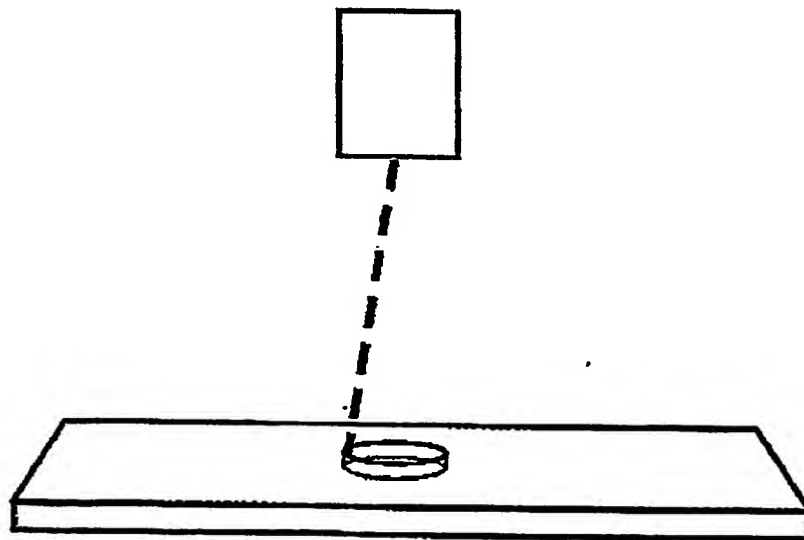


Fig. 15

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